

CLAIMS:

- Sub 7¹ 5*
1. A method of screening proteins or polypeptides which comprises forming a gene library and synthesising individual proteins, which can then be screened.
 2. A method as claimed in claim 1 wherein the individual proteins or polypeptides can be screened for (a) enzymatic protein modification and/or (b) binding to one or more other molecules/ligands and/or (c) binding or biological activity on cells or tissues.
 - 10 *B* 3. A method as claimed in ~~claim 1 or claim 2~~ wherein the gene library is derived from mRNA from one or more cells or tissues.
 - B* 4. A method as claimed in ~~claim 1 or claim 2~~ wherein the gene library encodes proteins or polypeptides comprising a library of variable molecules, such as fragments of antibody variable regions.
 - 15 5. A method as claimed in ^{*Claim 4*} ~~any one of claims 1 to 4~~ wherein the proteins or polypeptides are screened for binding to one or more other proteins or polypeptides from a cell or tissue.
 - 20 6. A method as claimed in claim 5 wherein any bound proteins or polypeptides from the cell or tissue are themselves screened for enzymatic modification.
 - B25* 7. A method as claimed in ^{*Claim 1*} ~~any one of claims 1 to 6~~ wherein the individual members of the gene library are initially distributed into one or more arrays whereby each gene is then expressed to generate one or more protein or polypeptide arrays.
 - B* 8. A method as claimed in ^{*Claim 7*} ~~any one or more of claims 1 to 7~~ wherein the proteins ^{*or*} polypeptides are immobilised onto a solid phase prior to screening.
 - 30 *B*

9. A method as claimed in claim 8 wherein the solid phase is a multi-well plate.

10. A method as claimed in claim 8 wherein the solid phase is a contiguous surface
5 such as a glass slide and wherein the proteins or polypeptides are immobilised at specific loci on the surface.

Claim 7
11. A method as claimed in ~~any one of claims 1 to 10~~ wherein the proteins or polypeptides are expressed by *in vitro* transcription and translation.

Claim 7
12. A method as claimed in ~~any one of claims 1 to 10~~ wherein the proteins or polypeptides are expressed by display on ribosomes.

Claim 7
13. A method as claimed in ~~any one of claims 1 to 10~~ wherein the proteins or polypeptides are expressed by display on bacteriophage.

14. A method as claimed in claim 12 wherein the proteins or polypeptides are indirectly immobilised onto a solid phase through annealing of mRNA in the ribosome display complex to complementary nucleic acid molecules located on the solid phase.

15. A method for screening proteins or polypeptides which comprises:

- (i) Generating a gene library, in the form of DNA, RNA, colonies or plaques;
- (ii) Converting the nucleic acid from each clone using *in vitro* translation to generate proteins or polypeptides;
- (iii) Dispensing aliquots of each protein or polypeptide into specific loci in multi-well plates or onto a solid phase to form protein or polypeptide arrays; and

- (iv) Bringing the arrays generated in (iii) into contact with one or more extracts from cells or tissues or with one or more cells or tissues *per se* in order to screen for protein or polypeptide modification or for binding to one or more molecules from the one or more extracts.

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16. A method for forming complexes of mRNA, ribosomes and proteins, comprising the addition of one or more molecules which binds to mRNA directly or indirectly to a mixture of said mRNA, ribosomes and proteins.

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17. A method as claimed in claim 16 wherein the one or more added molecules also bind to proteins within the complex of mRNA, ribosomes and protein.

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18. A method as claimed in ~~claim 16~~ or claim 17 wherein the one or more added molecules is/are a protein(s).

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19. A method as claimed in claim 18 wherein the added molecule is a bispecific antibody.

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20. A method as claimed in claim 18 wherein the added molecule binds to mRNA via a synthetic oligonucleotide which anneals to the mRNA.

21. A method as claimed in claim 18 wherein the added molecule binds to mRNA directly.

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22. A method as claimed in claim 20 wherein the synthetic oligonucleotide is attached to a biotin moiety and the added molecule is streptavidin

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23. A method as claimed in claim 21 wherein the added molecule is HIV tat (or a fragment thereof), Iron Regulatory Protein, La antigen or proteins from RNA viruses which bind to RNA.

24. A method as claimed ^{in claim 16} ~~in any one of claims 16 to 23~~ further comprising the addition of one or more molecules which inhibit initiation of mRNA translation.

25. A method as claimed in claim 24 wherein the molecule added to inhibit initiation is Signal Recognition Protein, cytomegalovirus gp48 (or a fragment thereof) or proteins from RNA viruses which bind to RNA

26. A method for screening proteins or polypeptides which comprises:

- (i) Generating a gene library in the form of DNA, RNA, colonies or plaques;
- (ii) Carrying out *in vitro* translation to produce proteins or polypeptides, wherein the translation reaction includes addition of molecules which bind to mRNA directly or indirectly and facilitate formation of complexes of mRNA, ribosomes and proteins or polypeptides.

27. A method for directly selecting a biological phenotype, comprising carrying out a method as defined in any one of claims 1 to 26, followed by bringing one or more of the displayed proteins or polypeptides into association with a target cell to allow binding of the one or more proteins or polypeptides to the cell.

28. A method as claimed in claim 27 wherein binding to the target cell results in an alteration to the target cell which then permits isolation of the target cell and recovery of genes encoding the displayed protein or polypeptide.

29. A method as claimed in claim 27 wherein binding to the target cell results in alteration to the target cell resulting in the production or cessation of production of one or more molecules from the target cell which then permits recovery of genes encoding the displayed protein or polypeptide.

30. A method as claimed in claim 29 wherein one or more of the molecules produced by the target cell binds to one or more of the components of the complex.

5 31. A method as claimed in claim 29 wherein one or more of the molecules produced by the target cell is required for the subsequent viability of a living microorganism.

10 32. A method as claimed in claim 31 wherein the microorganism is a bacteriophage or a bacteria.

15 33. A method as claimed in claim 29 wherein one or more of the molecules produced by the cell is released from the cell and in turn results in the release of other molecules from liposomes.

34. A method as claimed in claim 28 wherein the alteration to the cell results in the appearance or disappearance of a cell surface marker on the cell.

20 35. A method as claimed in claim 30 wherein the molecule derived from the target cell is a RNA binding polypeptide such as HIV tat.

25 36. A method as claimed in claim 31 wherein the molecule derived from the target cell is a bacteriophage polypeptide or an antibiotic or a drug resistance enzyme/factor or an essential nutrient.

30 37. A method of screening proteins or polypeptides which comprises carrying out a method as defined in any one of claims 1 to 26, followed by bringing one or more of the synthesised proteins or polypeptides into the vicinity of a modified ligand which binds to a receptor on the surface of a cell or tissue to label the synthesised proteins or polypeptides on the cell/tissue surface.

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38. A method as claimed in claim 37 wherein the displayed proteins or polypeptides are used to isolate cell surface molecules.

39. A method as claimed in claim 37 ~~or claim 38~~ wherein the ligand is a protein and is modified by attachment to one or more other molecules which can function as all or part of a label or can initiate a labelling reaction.

40. A method as claimed in claim 39 wherein phospholipase C is attached to the ligand and liposomes are added after binding of the ligand to the cell or tissue surface such that phospholipase C results in release of the liposome contents.

41. A method as claimed in claim 40 wherein the liposome contains streptavidin, HIV tat, signal recognition particle (SRP), an antibody (or fragment thereof), a specific mRNA or protein binding molecule, F pilus or nickel.

42. A method as claimed in claim 39 wherein horseradish peroxidase, beta-galactosidase or porin is attached to the ligand.

43. A method for labelling and isolation of one or more molecules on the surface of a cell or tissue which comprises:

- (i) binding a modified ligand on the cell/tissue surface and, in parallel, binding a library of synthesised proteins/polypeptides;
- (ii) initiating a labelling reaction from the ligand whereby synthesised protiens/polypeptides in the vicinity of the ligand become labelled;
- (iii) using the label on the synthesised proteins/polypeptides to recover genes encoding the synthesised proteins/polypeptides; and

- (iv) using the recovered genes to regenerate individual synthesised proteins/polypeptides in order to isolate molecules on the cell/tissue surface which are bound by the synthesised proteins/polypeptides.

5 44. A method for isolating a gene encoding a protein or polypeptide which binds to a ligand, which comprises carrying out a method as defined in any one of claims 1 to 26, and bringing the synthesised proteins or polypeptides into association with the ligand such that binding between the proteins or polypeptides and the ligands can occur, which in turn allows for recovery of genes encoding the synthesised proteins or polypeptides.

10 45. A method as claimed in claim 44 wherein recovery is achieved by means of molecular tags provided on one or both of the synthesised proteins/polypeptides and ligands.

15 46. A method as claimed in claim 44 or claim 45 wherein the ligand is itself a protein or polypeptide produced according to a method as defined in any one of claims 1 to 26.

20 47. A method as claimed in claim 46 wherein both proteins or polypeptides are encoded by the same mRNA molecule.

25 48. A method as claimed ^{in claim 45} ~~in any one of claims 44 to 47~~ wherein the molecular tag is HIV tat which binds to the mRNA encoding the protein or polypeptide.

49. A method as claimed ^{in claim 45} ~~in any one of claims 44 to 47~~ wherein the molecular tag is a polyhistidine peptide.

50. A method as claimed in claim 46 ~~or claim 47~~ wherein the molecular tag on one protein or polypeptide is HIV tat and on the other protein or polypeptide is a polyhistidine peptide.

51. A method as claimed in claim 44 wherein the individual proteins or polypeptides associate to provide an enzyme activity.

52. A method as claimed in claim 51 wherein the enzyme activity is beta-galactosidase or phospholipase C.

53. A method as claimed in claim 46 ~~or claim 47~~ wherein the individual proteins or polypeptides associate to provide a transcriptional activator or repressor

54. A method as claimed in claim 46 ~~or claim 47~~ wherein the individual proteins or polypeptides associate within a living microorganism to provide a positive or negative selection for that microorganism.

55. A method for isolating a gene encoding a protein which binds to a ligand, which comprises generating a library of displayed proteins or polypeptides as defined in any one of claims 1 to 26, generating a library of ligands, wherein the library of proteins or polypeptides and the library of ligands are each provided with a molecular tag, which may be the same or different, bringing the library of proteins or polypeptides and the library of ligands into association, and isolating those proteins or polypeptides which bind to ligands by one or more steps of isolation of said molecular tags.

56. A method as claimed in claim 55 wherein the genes encoding those proteins or polypeptides which bind ligands are recovered in order to identify the displayed proteins.

57. An array or library of proteins/polypeptides produced according to a method as defined in any one of claims 1 to 26.

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